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Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF
CORRECTION UNDER 37 CFR 1.322
Docket No. UF-174D4
Patent No. 7,192,730

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : David Michael Young
Issued : March 20, 2007
Patent No. : 7,192,730
For : Thermostable Proteolytic Enzymes and Uses Thereof in Peptide and Protein Synthesis

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Certificate
MAY 23 2007
of Correction

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:

Column 9, line 16:

"ofthis band"

Application Reads:

Page 14, line 18:

--of this band--

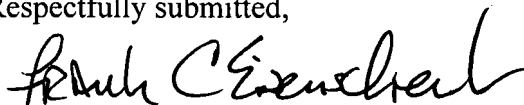
MAY 23 2007

Column 9, line 55:"In c vs r^2 "Page 15, line 15:--ln c vs r^2 --.

A true and correct copy of pages 14 and 15 of the specification as filed which support Applicant's assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/jps

Attachments: Copy of pages 14 and 15 of the specification

- 5 d. The pooled fractions from (c) are concentrated by membrane filtration and applied to a 2.6 x 60 cm column of Superdex-200 equilibrated with 0.1 M tris-HCl, 0.1 NaCl, pH 7.5.

Throughout the above ion-exchange steps (a-c), the chromatographic profiles reveal 2 prominent protein peaks that display BAPNA anilidase activity, together with other BAPNA-positive peaks in much lower amounts that are successively eliminated with each column step. The last step (gel filtration) yields 2 well-separated protein fractions that represent approximately 80% and 15% of the anilidase activity present in the original cell sonicate. The most abundant of these 2 proteins is the one used for all of the studies described below. It emerges from the Superdex-200 column with an apparent molecular weight of about 110,000 as judged from its partition coefficient determined with standard gel filtration molecular weight marker proteins. Polyacrylamide gel electrophoresis (SDS-PAGE) yields a single sharp band under reducing conditions. The estimated molecular weight of this band is approximately 81,000. The yield is approximately 1 mg of pure protein from 100 g wet cells.

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Example 2 – Extinction Coefficient, Apparent Partial Specific Volume, and Molecular Weight of Serine Protease of the Subject Invention

The protein was hydrolyzed (constant boiling HCl) for 18, 22, 24 and 26 hours. From absorbance measurements (280 nm) and the methods of Edelhoch (Edelhoch, H. "Spectroscopic Determination of Tryptophan and Tyrosine in Proteins." *Biochemistry* 6:1948-1954, 1967), the extinction coefficient was calculated to be $1.31 \text{ ml mg}^{-1} \text{ cm}^{-1}$.

Sedimentation equilibrium measurements utilized a Beckman Model E ultracentrifuge equipped with a split-beam scanner and multiplexer for visualization of two centrifuge cells during the same run. The high speed method of Yphantis (Yphantis, D.A. "equilibrium Ultracentrifugation of Dilute Solutions," *Biochemistry* 3:294-303, 1964) was employed together with the methods of Edelstein and Schachman for simultaneous measurement of the partial specific volume (Edelstein, S.J. and Schachman, H.K. "The

MAY 23 2007

5 Simultaneous Determination of Partial Specific Volumes and Molecular Weights with
Microgram Quantities." *J. Biol. Chem.* 242:306-311, 1967). One cell contained protein
dialyzed thoroughly against 0.1 M tris-HCl, pH 7.5, in H₂O and the second cell contained
the enzyme in the same buffer with 99% D₂O as solvent (densities of the buffer solutions
were measured pycnometrically). Centrifugation (20,000 RPM, 23.5EC) yielded a molecular
10 weight of approximately 81,500 and an apparent partial specific volume (Casassa, E.F. and
Eisenberg, H. "Thermodynamic Analysis of Multicomponent Solutions." *Adv. Prot. Chem.*
19:287-393, 1964) of 0.789 ml/g. This is a surprisingly high value for the specific volume of
a protein and it implies a larger than expected Stokes radius, which may explain why the
protein emerges earlier upon gel filtration than would be anticipated for a protein of
15 molecular weight of 81,500. Plots of $\ln c$ vs r^2 were strictly linear—a feature that indicates
size homogeneity. The close similarity of the molecular weight to that obtained by SDS-
PAGE indicates that the protein has a single polypeptide chain structure.

Example 3 – Stability of Enzymic Activity at High Temperature

20 For all kinetic experiments at high temperatures, sodium phosphate (0.025 M) was
used as a buffer. The temperature coefficient of this buffer is so small that slight changes in
pH with temperature do not significantly affect the kinetic data.

To assess stability of the enzyme at high temperature, a solution of the protein in the
above buffer, pH 7.0, was incubated at 82.0 +/- .05°C. Aliquots were removed at hourly
25 intervals up to 8 hr, and initial velocities were measured (BAPNA as substrate, Varian 2290
recording spectrophotometer) at 25.0° +/- .05°C (Erlanger, B.F., Kokowski, N. and Cohen,
W. "The Preparation and Properties of Two New Chromogenic Substrates of Trypsin." *Arch.*
Biochem. Biophys. 95:271-278, 1961). No decrease in enzyme activity was observed over
this time period.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,192,730
APPLICATION NO.: 10/825,921
DATED : March 20, 2007
INVENTOR : David Michael Young

Page 1 of 1

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 9,

Line 16, "ofthis band" should read --of this band--.

Column 9,

Line 55, "ln c vs r²" should read --ln c vs r²--.

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